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# Characteristics of Kombucha fermentation on medicinal herbs from Lamiaceae family

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## Abstract

Kombucha is a traditional beverage obtained by fermenting sweetened tea with tea fungus. Because of many purine derivatives, sweetened black or green tea has been almost the only recommended medium for preparing Kombucha. The aim of this paper was testing the possibility of obtaining Kombucha from medicinal herbs belonging to Lamiaceae family: lemon balm, thyme, peppermint and sage. During fermentation, the changes in chemical and microbiological parameters were determined, as well as main metabolites in Kombucha beverages. Both in small and traditional bioreactors, the most effective herb was lemon balm, then peppermint and thyme. Because Kombucha beverage with optimal consuming acidity was obtained in a shorter time compared with traditional medium, lemon balm and peppermint tea could be alternative medium for Kombucha fermentation, while sweetened sage tea is not adequate medium because the process is prolonged for four days. Yeasts number was from 6.3 to 7.3 log cfu units, and acetic acid bacteria from 5 to 7 log cfu. Acetic acid is dominant organic acid in all beverages which content was from 40% to 81% of titratable acidity, and the ethanol content was less then 1%.

**Key words:** Kombucha; Lamiaceae herbs; fermentation parameters

## Introduction

Among many traditional fermented foods, Kombucha is a popular beverage which originated in Manchuria and then was spread to Russia, Germany and the rest of the world. Slightly sweet, carbonated, acidic tea beverage is obtained by fermentation of sweetened black tea with „tea fungus“. This beverage is benefitial for human health: for metabolic disease, arthritis, constipation, curing cancer etc. [1, 2, 3]. This effects have not be proven scientifically yet, but could be attributed to the presence of gluconic acid, glucuronic acid, vitamins, aminoacids, micronutrients produced during fermentation etc. [4].

The tea fungus is a symbiotic culture of acetic acid bacteria (*Acetobacter aceti, Acetobacter pasteurianus, Gluconobacter oxydans*) [3, 5] and yeasts (*Saccharomyces* sp., *Zygosaccharomyces kombuchaensis., Torulopsis* sp., *Pichia* sp., *Brettanomyces* sp.) [3, 6]. Sucrose as carbon source in the cultivaton medium is hydrolyzed by the enzyme invertase from tea fungus yeasts. The yeasts ferment glucose and fructose to ethanol, which is then oxidized by acetic acid bacteria to acetic acid. This is the main metabolic path of Kombucha fermentation, and acetic acid, ethanol and gluconic acid are the main tea fungus products [7, 8]. Other components present in Kombucha beverage are fructose, ethyl-gluconate, oxalic acid, saccharic acid, keto-gluconic acids, carbonic acids as well as tea components (catehins, theaflavins, flavonols etc.) and cells metabolites (invertase, amylase, other oxidative enzymes etc.) [3, 9].

Kombucha is typically prepared by fermenting tea sweetened with sucrose (50-100 g/L) and inoculated with previously fermented liquid tea broth at level of 10-20% or tea fungus pellicle. The substrate is incubated statically under aerobic conditions, usually for 10-

12 days at 20-28 °C [7, 8, 10]. The fermentation length could be different, even up to 60 days [11], and in that case the obtained beverage has mild vinegar taste. But, to obtain pleasantly sour beverage, fermentation should be terminated when titratable acidity content reached 4-4.5 g/L, which is confirmed by longtime consumers of the Kombucha beverage. This value of titratable acidity is called optimal consuming acidity [12].

Tea in medium for cultivation provides necessary nitrogen sources (purine derivatives: caffeine and theophylline) for tea fungus culture. Regardless of the content of caffeine in green tea (about 5%) which is higher than in black tea (2%) and provides much higher nitrogen amount for tea fungus culture [13], black tea is a traditional and dominant source for Kombucha fermentation. Although Hoffmann (1998) noticed that some herbal teas can not be used as alternative nitrogen sources [13], Cvetković (2008) obtained Kombucha beverage from echinacea tea (*Echinacea purpurea* L.) and winter savory tea (*Satureja montana* L.) in shorter time compared with a traditional beverage [12].

Medicinal herbs from Lamiaceae family have a wide range of biological and pharmacological activities, so they are used for improving the flavour and organoleptic properties of different types of food. Also, their essential oils have antimicrobial, spasmolytic, carminative, anticancerogenic and other activities [14]. With respect to this, the possibility of obtaining Kombucha beverage by using medicinal herbs from Lamiaceae family (lemon balm, peppermint, thyme and sage) as the only nitrogen source, are reported in the present study. Tested chemical and microbiological parameters during fermentation, as well as the content of some metabolites in beverages, will be compared with the traditional beverage made from black tea. If those beverages can be obtained for acceptable time in small bioreactors, the experiments will be done in traditional bioreactors, too. In that way, we will see how the increasing volume influences the fermentation process.

## Materials and Methods

*Tea fungus*

The fermentation was performed by using a local tea fungus culture, for which previous investigations [15] showed that it contained at least five yeast strains (*Saccharomycodes ludwigii, Saccharomyces cerevisiae, Saccharomyces bisporus, Torulopsis* spp. and *Zygosaccharomyces* spp.) and two bacterial strains of the *Acetobacter* genera.

*Fermentation conditions*

All experiments were performed on a sweetened tea made by dissolving 70 g of sucrose in 1 L of tap water. To the boiled water the following amounts of tea were added: 2 g/L black tea (*Camelia sinensis* L.), 5 g/L lemon balm (*Melissa officinalis* L.), 5 g/L thyme (*Thymus serphyllum* L.), 5 g/L sage (*Salvia officinalis* L.) (the concentration for consuming, not for mouthwash) and 6 g/L peppermint tea (*Mentha piperita* L.) (Fructus, Bačka Palanka, Serbia) and removed by filtration after 15 min. After cooling to room temperature, the tea was inoculated with 10% of the fermentation broth from the previous fermentation of black tea obtained under the same conditions. Small bioreactors (volume 0.72 L, diameter 8 cm) and traditional bioreactors (volume 5 L, diameter 16 cm) were filled in with 0.33 L and 3.3 L of the inoculated liquid phase. The bioreactors were covered with cheesecloth, and the fermentation was monitored at 28±1 $^{o}$C.

*Sampling*

A sampling of the fermentation broth was performed daily; each bioreactor was sampled only once in order to avoid the potential contamination. During fermentation pH

value, titratable acidity, number of yeasts and acetic acid bacteria were determined. In the finished beverages, acetic acid and ethanol content was determined.

*Methods of Analysis*

The pH values were measured using an electronic pH meter (HI 9321, HANNA Instruments) calibrated at pH 4.0 and 7.0.

The titratable acidity was determined according to O.I.V. (2008) [16]. After removing $CO_2$ from the fermentation broth, a 20-ml aliquot was taken and titrated with 0.1 mol/L of NaOH. The titratable acidity was expressed in grams of acetic acid per liter of the sample.

The content of ethanol and acetic acid was determined by HPLC (instrument Pharmacia LKB; RI detector: SP6040 Spectra-Physics; column Aminex HPX-87H, 300 x 7,8 mm (BioRad)). The conditions of determination were: flow of mobile phase 0,6 mL/min; mobile phase 5 mM $H_2SO_4$, using methanol as internal standard.

The content of ethanol and acetic acid was determined by commercial enzymatic tests (Megazyme, Ireland), too.

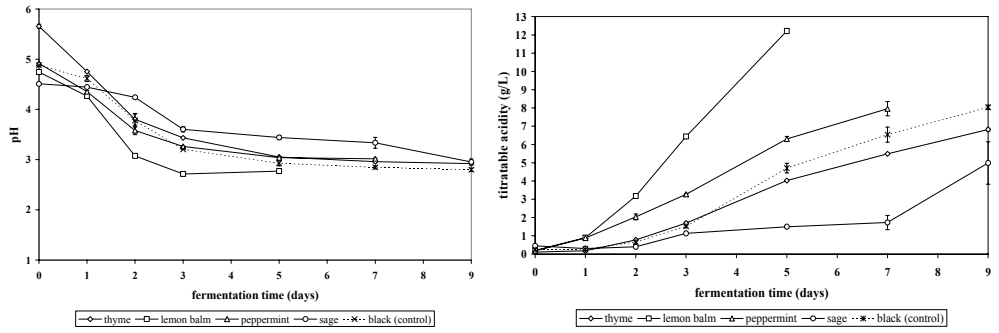
Total counts of cells of yeasts and acetic acid bacteria in the fermentation broth were determined by a plate pour method. For yeasts, the medium was Sabouraud-4% Maltose Agar (Merck, Darmstadt, Germany) with the addition of 50 mg/L of chloramfenicol (Sigma-Aldrich, St. Louis, USA) as an antibiotic, and the plates were incubated for 72 hours at 28 °C. The medium for determining the total count of acetic acid bacteria was Yeast Peptone Mannitol Agar (Difco, Detroit, USA), containing 500 mg/L cycloheximide (actidione; Sigma-Aldrich, St. Louis, USA) to inhibit the yeasts growth. The incubation at 28 °C lasted for 5-7 days.

All experiments were performed in duplicate, under the same conditions, while each quantity was measured three times. The obtained values used for further processing are the averages of all the measurements and are presented as mean ± standard deviation.

## Results and Discussion

*Changes in chemical and microbiological parameters in small bioreactors*

The average changes of pH and titratable acidity during fermentation in small bioreactors are presented in Figure 1.




(a) (b)

**Fig 1.** The changes in pH values (a) and titratable acidity (b) during Kombucha fermentation on medicinal herbs (thyme, lemon balm, peppermint, sage) as well as black tea (control) in small bioreactors

The pH value of the sweetened tea from Lamiaceae herbs, as well as black tea (Fig 1, a), was approximately 7, and it dropped to about 4.5-5.66 immediately after the inoculation. In first two days the pH value decreased by 0.5-0.9 units per day and on the next day a

decrease was only 0.3 units. After this period, the pH value changed insignificantly and reached 2.82-2.95 at the end of the process. For fermentation broth with sage, decreasing in pH value started after two days, reached 3.6 on the third day of the process and slowly decreased to 2.95 in the next six days. The changes in pH values of the fermentation broth were similar for both, control Kombuha as well as Kombucha with Lamiaceae herbs. The same pH trend was observed by some other authors who used similar cultivation conditions [1, 8, 17]. These authors noticed changes in pH values from about 6-7 at the beginning of the process, rapidly decreasing in the following three to five days and keeping constant value at about 2-3 units until the end of the process.

Titratable acidity content (Fig 1, b) increased from the beginning till the end of the fermentation process for all Lamiaceae herbs except sage. The highest production of acids was in the fermentation broth with lemon balm tea where optimal consuming acidity (about 4 g/L) was obtained for less than three days. This value for peppermint tea was reached probably on the fourth day and for thyme on the fifth day. In case of sage only 0.4 g/L of acids was synthesized between three and seven days of fermentation, and the process was terminated after 9 days (titratable acidity was 4.95 g/L). Compared with the control Kombucha, where optimal consuming acidity was reached after five days, using sweetened lemon balm and peppermint tea process is shortened for one or two days. For similar fermentation conditions, Liu et al. (1996) obtained optimal consuming acidity (4 g/L) after six days [6], while Sievers et al. (1995) obtained the same acidity after nine days of fermentation [7]. The production of acetic acid is limited during prolonged tea fungus cultivation (17 and 60 days) [10, 11]. This can be explained by the fact that high medium acidity and lower amounts of sugar inhibit the fermentation activity of yeasts and the production of ethanol (and indirectly the production of acetic acid).

As it is obvious, differences in titratable acidity between samples are more expressed than changes in pH, because of buffer character of the Kombucha beverage. So, titratable acidity was used as a critical parameter which determines the end of Kombucha fermentation instead of pH value [18]. Although the optimal consuming acidity is defined as a critical parameter, in tested Lamiaceae herbs, the fermentation was terminated few days after reaching this value. This is because some of these herbs, for the first time, were used as medium for Kombucha fermentation and it was unknown which fermentation pattern (chemical and microbiological) will be in the prolonged period, as well as which will be compared with traditional Kombucha fermentation (with black tea).

The average changes in microbiological parameters during fermentation in small bioreactors are presented in Figure 2.

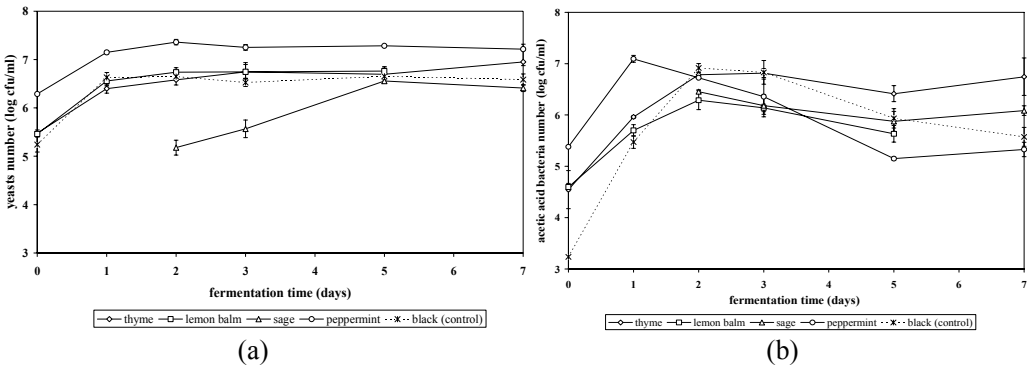



**Fig 2.** The changes in the yeasts (a) and acetic acid bacteria number (b) during Kombucha fermentation on medicinal herbs (thyme, lemon balm, peppermint, sage) as well as black tea (control) in small bioreactors

At the beginning of the process (after inoculation), the differences between the samples were noticeable both in number of yeasts and acetic acid bacteria, because the applied inoculums did not have the same number of cells. The least number of cells had the medium with sage (less then 3 log units in the first two days). This is probably because of the well known antiseptic characteristics of sage [19, 20] which can inhibit tea fungus cells. After that, the number of cells increased, probably because antimicrobial substances from the sage were spent and some tea fungus cells stayed live and capable of multiplying. So, at the end of the process in the medium with sage, the number of yeasts reached 6.4 log cfu units, and acetic acid bacteria 5.7 units. For other medium, the changes in the yeast number are similar (Fig 2, a). After increasing in the first 24 hours for one log unit, the number of yeasts was uniform until the end of the process (on range 6.4 for sage to 7.2 log units for peppermint). The medium with lemon balm and thyme had a similar curve pattern as well as the number of yeasts in a traditional medium (with black tea).

Higher differences between the samples are noticed for acetic acid bacteria number during fermentation (Fig 2, b). After rapidly increasing in the first 24h to 7.1 log cfu units (for the medium with peppermint tea) or in 48 hours to 6.29-6.92 log units (for other samples), the number of cells decreased until the fifth day of the process for 0.4-1.85 log units and then slightly increased until the end of the process when number was from 5.32 log units for peppermint to 6.74 log units for medium with thyme. Although a traditional medium had the least number of acetic acid bacteria after inoculation (3.24 log units), this number is at the same range as in other samples in the following six days.

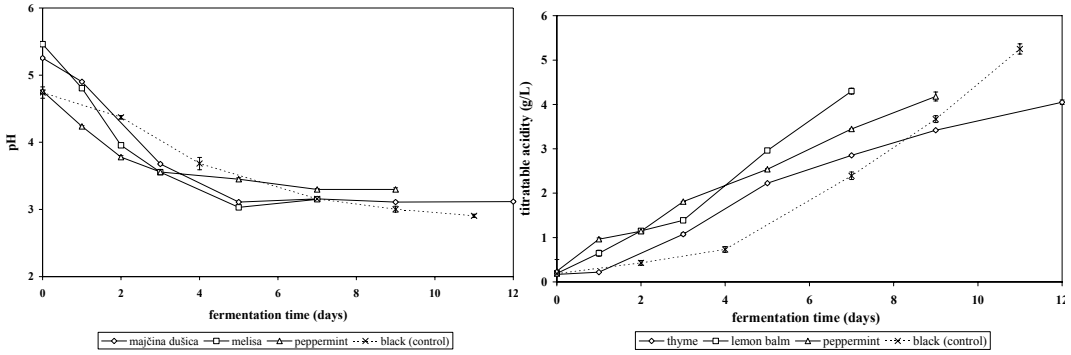
Teoh et al. (2004) reported that during Kombucha fermentation, the viable population of yeasts followed a standard growth curve pattern, in which yeast grew exponentially for up to 8-10 days, reaching 7 log units, dying off as nutrients became limiting and the pH decreased [9]. The same maximal yeast number is reached by Chen and Liu (2000) between 6 and 14 days and after that number decreased until the end of the process (30 days) as a consequence of nutrients absence. Similar changes are present for acetic acid bacteria number with maximum 4 log units [11]. After achieving maximal 7 log units of yeasts and acetic acid bacteria, Sreeramulu et al. (2000) obtained no uniform changes in the cells number [1].

Generally, the same differences in chemical parameters, the process duration and cell counts in Kombucha beverages obtained in different studies are expected because of using inoculums (Kombucha cultures) from different locations. The variations could be due to geographic, climatic and cultural conditions as well as on local species of wild yeasts and bacteria [9, 21] or possibly from cross-contamination between cultures.

The same as for chemical parameters and for microbiological parameters the differences between the medium with Lamiaceae herbs and control Kombucha were not obvious. This means that tested medicinal herbs can be used as an alternative medium for Kombucha fermentation, especially sweetened lemon balm and peppermint tea because the process is shortened compared with a traditional medium. Sweetened thyme tea, which enables the same process duration as a traditional medium, can also be used for Kombucha fermentation. Using medium with the sage process is prolonged for four days which means that sweetened sage tea is not adequate as an alternative medium. Because of that, further experiments in traditional bioreactors were not performed with sweetened sage tea.

*Changes in chemical and microbiological parameters in traditional bioreactors*

The average changes of pH and titratable acidity during fermentation in traditional bioreactors are presented in Figures 3.




(a) (b)

**Fig 3.** The changes in pH values (a) and titratable acidity (b) during Kombucha fermentation on medicinal herbs (thyme, lemon balm, peppermint, sage) as well as black tea (control) in traditional bioreactors

pH values (Fig 3, a) in traditional bioreactors were changed in the same way as in small bioreactors (Fig 1, a), but decrease stopped on the fifth day of the process and stayed constant until the end (about 3 pH units). This indicated that the process in traditional bioreactors could be prolonged compared with small ones. This fact is confirmed by changes in titratable acidity (Fig 3, b). As in small bioreactors (Fig 1, b), in traditional bioractors the highest production of acids was for the medium with lemon balm, where about six days are needed to obtain the optimal consuming acidity. The next medium according to the process duration was the medium with peppermint (nine days) and then thyme (twelve days). Compared with control Kombucha (optimal consuming acidity was obtained after nine days), as in the case of small bioreactors, the process is shortened for the medium with lemon balm even to three days, and for the medium with peppermint and thyme, the process duration is the same.

It is obvious that, with the increase of the bioreactor volume and medium volume in it, the fermentation process is prolonged for about four days for all medicinal herbs as well as for control Kombucha. This phenomenon can be explained with the reduction of available oxygen to acetic acid bacteria in the case of higher volumes. Namely, in small bioreactors, acetic acid bacteria had more available oxygen, because a free space above the fermentation liquid is higher (about 55% compared to the medium volume). On the contrary, in traditional bioreactors, a free space is 34%. The other fact that can cause the prolongation of the process is cellulosic pellicle, which is larger and heavier in traditional than in small bioreactors and makes the exchange of gases, availability of oxygen and appearance of $CO_2$ from the system more difficult [18].

Malbaša et al. (2006) determined pH in bioreactors with 4L of medium and obtained a constant value of 3.8 after nine days of fermentation [22]. Goicochea et.al. (2000) determined titratable acidity in 2L of the fermentation broth [23]. A significant synthesis of acids started after five days of fermentation, and reached about 5 g/L on the seventh day. The process was shortened compared with Lamiaceae herbs because the volume of the medium was less.

According to Hoffmann (1998), herbal teas (peppermint, linden, hazelnut etc.) can not be used as an alternative medium for Kombucha fermentation because of the absence of purine derivatives [13]. They can be used only in combination with black tea, which would provide necessary amounts of nitrogen sources. Regardless, in this paper Kombucha beverage with a satisfactory amount of acids can be successfully obtained from Lamiaceae herbs, even sage, but for a longer time. Similar, Cvetković (2008) obtained a Kombucha beverage from Ehinacea tea (herba and radix) in a shorter time compared with a traditional beverage. Total acids for an Echinacea beverage were about 5 g/L which is for 2 g/L higher compared with a

traditional one. A similar content of acids was obtained for winter savory tea which can also replace black tea as a medium for cultivation. That confirmed the possibility of using some alternative medicinal herbs as substrates for Kombucha fermentation [12].

The changes in microbiological parameters in small bioreactors (Fig 2) showed that a periodical decrease in cells number during fermentation did not influence the process duration and their prolongation. So, the number of yeasts and acetic acid bacteria in traditional bioreactors was determined on the day when the fermentation was terminated (Table 1).

**Table 1.** Microbiological parameters at the end of the fermentation process on medicinal herbs in traditional bioreactor as well as black tea (control)

| Herb (tea) | Day of fermentation | Cells number (log cfu/mL) | |
|---|---|---|---|
| | | Yeasts | Acetic acid bacteria |
| Lemon balm | 5 | 7.17± 0.02 | 6.94± 0.03 |
| Peppermint | 9 | 7.13± 0.04 | 5.69± 0.13 |
| Thyme | 12 | 5.64± 0.02 | 5.4± 0.01 |
| Black | 7 | 6.59± 0.2 | 5.57± 0.12 |

Yeasts number in Kombucha beverages obtained in traditional bioreactors was in the range of 5.64-7.17 log cfu units, which is similar as in small bioreactors. The exception is the medium with thyme with a less number for 1.3 log units at the end of the process. As for acetic acid bacteria number, for the medium with thyme, as well as for traditional medium, the number was less for about one log unit, while for other medium the number was in the same range as in small bioreactors. Present differences in the cells number did not affect the fermentation length. Namely, as in small bioreactors, the most efficient was the medium with lemon balm, then with peppermint and thyme which had the same duration as a traditional medium (Fig 3, b).

*Acetic acid and ethanol content in Kombucha beverages*

Main metabolites (ethanol and acetic acid) of Kombucha fermentation in small bioreactors at the end of the process were determined by two methods (HPLC and enzymatic tests) and the results are presented in Table 2.

**Table 2.** The content of ethanol and acetic acid (g/L±sd) in Kombucha beverages from different medicinal herbs, as well as black tea (control) determined by two methods

| Herb (tea) | Lemon balm | | Peppermint | | Thyme | | Black | |
|---|---|---|---|---|---|---|---|---|
| Method | HPLC | enzymatic | HPLC | enzymatic | HPLC | enzymatic | HPLC | enzymatic |
| Ethanol | 2.86 | 4.76±0.057 | 4.4 | 5.23±0.198 | 0.926 | 1.18±0.03 | 4.07 | 4.67±0.198 |
| Acetic acid | 1.64 | 1.83±0.025 | 6.49 | 5.34±0.002 | 2.752 | 2.26±0.018 | 4.74 | 4.60±0.198 |

As it is expected, acetic acid is a dominant organic acid in Kombucha beverages. In Kombucha beverage with peppermint tea, acetic acid made even 81% of titratable acidity on the seventh day of the process determined by HPLC method, while it made 75% by an enzymatic method. The content of acetic acid in a beverage with thyme was 40% of total acids, as determined by HPLC, and the differences between the methods were 17%. Regardless of the low concentration of acetic acid in a beverage with thyme, it can not be concluded that it is not a dominant acidic component in a beverage until all present acids are analyzed. Because of a very high titratable acidity on the fifth day of the process (12.21 g/L), the acetic acid content in a lemon balm beverage was determined on the second day. By both

methods, acetic acid made 50% of all acids in the beverage. In control Kombucha made from black tea, acetic acid made about 70% of total acids, which confirmed that this is a dominant organic acid. According to the results shown (Table 2), it is obvious that for acetic acid determination both methods are equally efficient.

Larger differences between the methods were observed during determination of ethanol in the medium with lemon balm, where 1.9 g/L more ethanol was determined by enzymatic method than by HPLC. For other alternative medium (with thyme and peppermint) a higher content of ethanol was obtained by enzymatic method, too. The least content of ethanol had Kombucha with thyme (1.18 g/L) and the highest Kombucha with peppermint (5.23 g/L), which can be connected with the highest number of yeasts during the whole process. The content of ethanol would probably be less if it was determined on the forth or fifth day when titratable acidity was obtained. The ethanol content (determined by enzymatic tests) in control Kombucha is similar as in lemon balm Kombucha (about 4 g/L). In those beverages the number of cells at the end of the process is similar, too. In Kombucha from thyme, the number of acetic acid bacteria was the highest, which can explain less ethanol content (more ethanol is oxidized to acetic acid by acetic acid bacteria). Higher differences between the methods are present during ethanol determination, but regardless of this, HPLC method has known a comparative advantage because it is clearly faster and more convenient, allowing more compounds to be quantified simultaneously [24].

Other authors used one of these methods for determination of main metabolites in a Kombucha beverage. So, Sievers et al. (1995) obtained about 3 and 4 g/L of acetic acid in two Kombucha beverages (made from different tea fungus) after 8 days of fermentation using enzymatic tests [7]. By HPLC, Chen and Liu (2000) marked acetic acid as dominant organic acid (62% of titratable acidity) [6], while Jayabalan et al. (2007) obtained only 1.5 g/L acetic acid after the same period (six days) [4]. But regardless of different concentration of acetic acid obtained in the same period in different studies, it is confirmed that this is a dominant organic acid in a Kombucha beverage.

As for the ethanol content Sievers et al. (1995) obtained less than 5g/L [7] after six days, while Reiss (1994) obtained 3.3 g/L by using enzymatic tests [10]. Using HPLC Liu et al. (1996) obtained 7.83 g/L [6] after six days. By gas chromatography, Chen and Liu (2000) obtained about 3 g/L after 10 days [11].

Although the results are discussed for the same fermentation period, deviations are presented both for acetic acid and ethanol determination because of using different tea fungus and fermentation conditions, as well as unequal amounts of carbon and nitrogen sources.

## Conclusions

In small bioreactors Kombucha could be obtained successfully from lemon balm, peppermint and thyme tea. In case of lemon balm and peppermint tea the process is shortened for one or two days compared with a traditional medium (made from black tea). The same process duration is for the medium with thyme, while sweetened sage tea is not recommended because the process is prolonged for four days. In traditional bioreactors, the most efficient was the medium with lemon balm, too, when the process is shortened for three days compared with a traditional one. The same process duration is for the medium with peppermint and thyme tea. That means those medicinal herbs can successfully be used as the only substrate (not in combination) for Kombucha fermentation instead of black tea. With increasing the medium and bioreactor volume, the fermentation process is prolonged for about four days for all tested mediums. The changes in yeasts and acetic acid bacteria number during fermentation (periodically decreasing in number), as well as less number in a traditional

bioreactor for some mediums, do not affect synthesis of acids and the fermentation length. Acetic acid is a dominant organic acid in almost all beverages.

## References


1. G. SREERAMULU, Y. ZHU, W. KNOL, Kombucha fermentation and its antimicrobial activity. *J. Agr. Food Chem*., 48(6), 2589-2594 (2000).
2. C. DUFRESNE, E. FARNWORTH, Tea, Kombucha and Health: A Review, *Food Res. Int*., 33, 409-421 (2000).
3. C.J. GREENWALT, K.H. STEINKRAUS, R.A. LEDFORD, Kombucha, the Fermented Tea: Microbiology, Composition, and Claimed Health Effects, *J. Food Prot*., 63(7), 976-981 (2000).
4. R. JAYABALAN, S. MARIMUTHU, K. SWAMINATHAN, Changes in content of organic acids and tea polyphenols during kombucha tea fermentation, *Food Chem*., 102, 392-398 (2007).
5. I. JANKOVIĆ, M. STOJANOVIĆ, Microbial and chemical composition, growth, therapeutical and antimicrobial characteristics of tea fungus, *Mikrobiologija*, 33, 25-34 (1994).
6. C.-H. LIU, W.-H. HSU, F.-L LEE, C.-C. LIAO, The isolation and identification of microbes from a fermented tea beverage Haipao, and their interaction during Haipao fermentation, *Food Microbiol*., 13, 407-415 (1996).
7. M. SIEVERS, C. LANINI, A. WEBER, U. SCHULER-SCHMID, M. TEUBER, Microbiology and Fermentation Balance in Kombucha Beverage Obtained from a Tea Fungus Fermentation, *Sys. Appl. Microbiol*., 18, 590-594 (1995).
8. P.J. BLANC, Characterization of Tea Fungus Metabolites, *Biotechnol. Lett*., 18, 139-142 (1996).
9. A.L. TEOH, G. HEARD, J. COX, Yeast Ecology of Kombucha Fermentation, *Int. J. Food Microbiol*., 95, 119 –126 (2004).
10. J. REISS, Influence of Different Sugars on the Metabolism of the Tea Fungus, *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, 198, 258-261 (1994).
11. C. CHEN, B.Y. LIU, Changes in major components of tea fungus metabolites during prolonged fermentation, *J. Appl. Microbiol*., 89, 834-839 (2000).
12. D. CVETKOVIĆ, Kombucha made from medical herbs - biological activity and fermentation, *PhD Thesis,* University of Novi Sad, Faculty of Technology, Novi Sad (2008) (in Serbian)
13. N. HOFFMANN, Basic Building Blocks, Nutrients and Growth Factors, What the Kombucha culture needs to survive, (http://www.kombu.de/nutrient.htm) (1998).
14. B. BOŽIN, N. MIMICA-DUKIĆ, N. SIMIN, G. ANACKOV, Characterization of the Volatile Composition of Essential Oils of some Lamiaceae Spices and the Antimicrobial and Antioxidant Activities of the Entire Oils, *J. Agric. Food Chem.*, 54, 1822-1828 (2006).
15. S.L. MARKOV, R.V. MALBAŠA, M.J. HAUK, D.D. CVETKOVIĆ, Investigation of Tea Fungus Microbe Associations. I. The Yeasts. *Acta Periodica Technologica*, 32, 133-138 (2001).
16. Compendium of international methods of wine and must analysis, International Organization of Vine and Wine (O.I.V.) Paris Total acidity (A 10), (MA-E-AS313-01-ACITOT) (2008).
17. G. BELLOSO-MORALES, H. HERNÁNDEZ-SÁNCHEZ, Manufacture of a beverage from cheese whey using a "tea fungus" Fermentation, *Rev. Latinoam. Microbiol*., 45(1-2), 5-11 (2003).
18. D. CVETKOVIĆ, S. MARKOV, M. DJURIĆ, D. SAVIĆ, A. VELIĆANSKI, Specific Interfacial area as a key variable in scaling-up Kombucha fermentation, *J. Food Eng*., 85, 387–392 (2008).
19. D.T. VELIČKOVIĆ, M.S. RISTIĆ, N.V. RANDJELOVIĆ, A.A. SMELCEROVIĆ, Chemical composition and antimicrobial characteristic of the essential oils obtained from the flower, leaf and stem of *Salvia officinalis* L. Originating from southeast Serbia, *J. Essent. Oil Res*., 14 (6), 453-458 (2002).
20. B. BOŽIN, N. MIMICA-DUKIĆ, I. SAMOJLIK, E. JOVIN, Antimicrobial and antioxidant properties of Rosemary and Sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils, *J. Agr. Food Chem*., 55 (19), 7879-7885 (2007).
21. P. MAYSER, S. FROMME, C. LEITZMANN, K. GRUENDER, The yeast spectrum of tea fungus Kombucha. *Mycoses*, 38 (7-8), 289-295 (1995).
22. R. MALBAŠA, E. LONČAR, M. DJURIĆ, LJ. KOLAROV, M. KLAŠNJA, S. MARKOV, Scale-up of Black Tea Batch Fermentation by Kombucha, *Food Bioprod. Process*., 84(C3), 193-199 (2006).
23. H.C. GOICOECHEA, D. ELUK, M.P. KUBESCHA, J.B. FERRARO, B.M. RODIL, H.F. MIGLIETTA, V.E. MANTOVANI, Probiotic Production: An Interesting Example for the Undergraduate Analytical Chemistry Laboratory, *Chem. Educator*, 5, 67-70 (2000).
24. D. LEFEBVRE, V. GABRIEL, Y. VAYSSIER, C. FONTAGNÉ-FAUCHER, Simultaneous HPLC Determination of Sugars, Organic Acids and Ethanol in Sourdough Process, *Lebensm.-Wiss. u.-Technol*., 35, 407-414 (2002).